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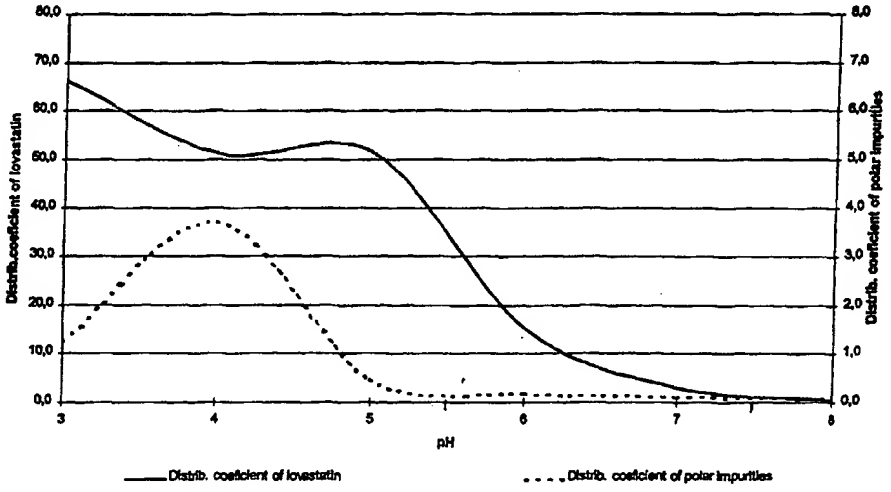


(57) Abstract

A process for the isolation and purification of HMG–CoA reductase inhibitors from a mycelium biomass is described, which process comprises: clarifying a mycelium broth and concentrating the clarified broth to a lower volume, acidifying of the concentrate to a pH value in the range of 4.5 to 7.5, followed by extracting the HMG–CoA reductase inhibitor with ethyl acetate, crystallization of the HMG–CoA reductase inhibitor from a water–miscible or water–soluble organic solvent, and crystallization of the HMG–CoA reductase inhibitor from an organic solvent having limited miscibility or solubility with water. The crystallization steps may also be reverse. The concept of a combination of the specified crystallization steps can also be used for the purification of a crude HMG–CoA reductase inhibitor.

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## TITLE OF THE INVENTION

**Process for the obtaining of HMG-CoA reductase inhibitors of high purity**

## Description

### *Background of the invention*

Lovastatin, pravastatin, mevastatin, simvastatin, their derivatives and analogs are known as HMG-CoA reductase inhibitors and are used as antihypercholesterolemic agents. They are produced by fermentation using microorganisms of different species identified as species belonging to *Aspergillus, Monascus, Nocardia, Amycolatopsis, Mucor* or *Penicillium* genus.

Purity of the active ingredient is an important factor for the manufacturing of a safe and effective pharmaceutical. The highest possible purity of the product is especially important if the pharmaceutical product should be taken for a longer period as it is the case in the treatment or the preventing of a high plasma cholesterol. The accumulation of the impurities from the pharmaceuticals of lower purity can cause many side effects during the medical treatment.

The processes for the isolation and purification of the antihypercholesterolemic agents disclosed in the earlier patent applications comprise different combinations of extraction, chromatography, lactonization and crystallization methods. The purity of the final product obtained by these procedures is lower than 99.6%. Obtaining the product of higher purity by use of these methods is possible, but the yield of the desired product is then unacceptably low for using those methods in a large industrial scale.

The isolation process disclosed in patent application WO 92/16276 provides the solution for obtaining HMG-CoA reductase inhibitors of purity higher than 99.5%, but the use of highly sophisticated industrial high performance liquid chromatography (HPLC) equipment is required. According to the WO 92/16276 the crude HMG-CoA reductase inhibitor of approximately 85% or higher purity is dissolved in an organic solvent or in a solution of organic solvent and water. The mixture is then buffered to a pH between 2 and 9 and placed on an HPLC column. After the HMG-CoA reductase inhibitor peak of interest is collected, a portion of solvent is removed and then water is added or alternatively two-thirds of the solvent mixture are removed to crystallize the HMG-CoA reductase inhibitor. At the end the purity of the product achieved by this process is really at least 99.5% with yield of approximately 90%.

## ***Summary of the invention***

The present invention relates to a new industrial process for isolation and purification of HMG-CoA reductase inhibitors of purity higher than 99.6% and preferably higher than 99.7 % from a fermentation broth. To achieve this goal an extensive study of the chemical compounds produced during the fermentation using the different species of microorganisms belonging to *Aspergillus, Monascus, Nocardia, Mucor, Amycolatopsis* or *Penicillium* genus, their chemical properties and their behavior in the different solvents at different pH was done. Thus, the aforementioned object was solved by the process of the present invention which comprises the following steps:

- clarifying a mycelium broth and concentrating the clarified broth to a lower volume,
- acidifying of the concentrate to a pH value in the range of 4.5 to 7.5, followed by extracting the HMG-CoA reductase inhibitor with ethyl acetate,

- optionally performing lactonization,
- performing crystallization of the HMG-CoA reductase inhibitor from a water-miscible or water-soluble organic solvent, and
- performing crystallization of the HMG-CoA reductase inhibitor from an organic solvent having limited miscibility or solubility with water.

## ***Detailed description of the invention***

Referring to the drawings,
Fig. 1 shows the dependency from the pH of the distribution coefficient of a HMG-CoA reductase inhibitor (lovastatin) and of impurities, respectively, in the ethyl acetate extraction step, and
Figs. 2, 3 and 4 show HPLC diagrams of samples of HMG-CoA reductase inhibitor after ethyl acetate extraction as a crude composition, after crystallization from a water-miscible or water-soluble organic solvent, and after further crystallization from an organic solvent having limited miscibility or solubility with water, respectively.

Since HMG-CoA reductase inhibitors are typically both intra- and extracellular products, it is not mandatory but preferable to dissolve them effectively from the mycelium into fermentation liquor. The method for dissolvation disclosed in patent application WO 97/20834 comprises treatment of fermentation broth with alkaline base to pH 11.5 and stirring for three hours. The WO 97/06128 teaches that dissolvation may be done with alkalifying of the fermentation broth to pH between 10 and 13. Also a temperature between 60 and 95 °C is applied. HMG-CoA reductase inhibitors may be very efficiently dissolved from the mycelium at a pH higher than 9, but too long exposure to so rigorous condition causes the degradation of ester bond between hydroxyl group on naphthalene skeleton and carboxylic acid. Equilibrium between

HMG-CoA reductase inhibitors and deacylated HMG-CoA reductase inhibitors shifts at more rigorous conditions to deacylated products. We have unexpectedly found out that the efficiency of dissolvation carried out in a temperature range of 10 to 40°C, preferably in the range of 18 to 25°C, such as room temperature, for less than one hour, preferably for less than half an hour, for example for about 10 minutes, at a pH between 9.5 and 13, most preferably between 9.5 and 11.5, is equal to the efficiency achieved by less economic and more time consuming methods carried out at higher temperatures described in earlier patent applications. The dissolvation may be carried out also at a pH lower than 9.5 and especially lower than 6, but the use of a huge amount of organic solvents is necessary in this case.

If this preferred embodiment of dissolvating the HMG-CoA reductase inhibitor has been carried out, the fermentation broth is subsequently treated with an acidifying agent, suitably with mineral acid, to adjust the pH value between 7.5 and 8.5. Suitable mineral acids are phosphoric, sulfuric and hydrochloric acid. HMG-CoA reductase inhibitors are stable in this range of pH and the fermentation broth can be also stored for a while after this step, if that is necessary or desired.

The mycelium is removed from the fermentation broth by means of appropriate separation steps, such as filtration and/or centrifugation. Filtration is preferred, and as a filtration technique beside classic filtration also micro-, ultra- and diafiltration may suitably be used. The clarified broth is then concentrated to a lower volume, most preferably five to ten times, by means of reverse osmosis or some other methods for lowering volume.

The acidification and ethyl acetate extraction step described in the following is a significant point of the purification process.

The said concentrate is acidified by an acidifying agent, suitably with mineral acid, to a pH value between 4.5 and 7.5. Mineral acids already mentioned above as examples can be used. Then, HMG-CoA reductase inhibitor is extracted from the said pH-adjusted concentrate with ethyl acetate. Extraction is suitably done by using a counter-current extraction column. The ratio between distribution coefficients of HMG-CoA reductase inhibitors and ethyl acetate soluble impurities is the highest at a pH value from 5.5 to 7.5 and especially at a pH value from 6.0 to 7.0, and a part of polar impurities is already removed at this step. The extraction carried out at pH value lower than 5.0, especially lower than 4, is more efficient, because of higher distribution coefficient of HMG-CoA reductase inhibitors, but it results in high level of polar impurities. The distribution coefficients of ethyl acetate soluble impurities are also high at that pH value, as is shown in Fig. 1. The extraction into ethyl acetate carried out at a pH value between 4.5 and 7.5, especially above 5.0 and in particular above 5.5, results in lower level of polar impurities because of their low distribution coefficients. Worse distribution of HMG-CoA reductase inhibitors from the concentrate into ethyl acetate at that pH value can be compensated with a longer counter-current extraction column.

If desired, the resulted ethyl acetate extract is then concentrated and HMG-CoA reductase inhibitor is lactonized optionally at this stage of the process. At pH between 5.5 and 7.5, the major part of the HMG-CoA reductase inhibitor is in free acid form. Therefore, the concentration and lactonization may be omitted if HMG-CoA reductase inhibitor is not used in the pharma-ceutical as a lactone. The lactonization is suitably done by contacting the HMG-CoA reductase inhibitor with catalytic amount of mineral or organic acid, most preferably trifluoracetic acid (TFA). The HMG-CoA reductase inhibitor which is optionally lactonized may then be directly crystallized from ethyl acetate, as will be described below. Alternatively, the ethyl acetate is removed, suitably by

vaporization, and a raw HMG-CoA reductase inhibitor product, which is optionally lactonized, is obtained.

The thus obtained raw HMG-CoA reductase inhibitor may then optionally be subjected to adsorption chromatography, preferably to reversed phase chromatography. As the mobile phase for adsorption chromatography, acetonitrile or lower alcohols such as methanol, ethanol or propanol, or a mixture of these solvents with water, can suitably be used. Preferably, the raw HMG-CoA reductase inhibitor is dissolved in pure acetonitrile or mixture acetonitrile/water with at least 30% volume/volume (v/v) of acetonitrile, and the resulting solution is placed on an adsorption chromatography column. The column packing include, but are not limited to stationary phases based on octylsilane, dimethylsilane, octadecylsilane, cyano-silane, polystyrenedivinylbenzene copolymer or acrylic polymer. Other typical stationary phase materials may also be used, for example silica, alumina, or the like. The adsorbed compounds are eluted with an appropriate mobile phase, such as acetonitrile/water gradient. The HMG-CoA reductase inhibitor peak of interest is collected and the mobile phase solvent is removed to crystallize the HMG-CoA reductase inhibitor. The purity of crystallized crude HMG-CoA reductase inhibitors is between 80% and 92% and depends on impurity profile in the fermentation broth. The optional adsorption chromatography may also be replaced by normal chromatography, flash chromatography, industrial HPLC, or by methods of extraction or crystallization.

The combined crystallization treatment which is peculiar according to the present invention will be described in more detail in the following.

More specifically, it comprises crystallization of the HMG-CoA reductase inhibitor from an organic solvent being water-miscible or water-soluble, and crystallization of the HMG-CoA reductase inhibitor from an organic solvent having a limited miscibility or solubility with water. The order of both

crystallizations may also be inverse. The property of the organic solvent of being either water-miscible or water-soluble, or having a limited miscibility or solubility with water is per se known to the man skilled in the art and is, for example, described in "Ullmann's Encyclopedia of Industrial Chemistry", Vol. A24, 5th edition (1993), pp. 437-505, incorporated herein by reference. In the meaning of the present invention, the term "water-miscible or water-soluble" shall refer to organic solvents which show essentially unlimited, preferably 100 % miscibility or solubility with water, and the term "limited miscibility or solubility with water" shall also include water-immiscible or water-insoluble organic solvents. Furthermore, the concept of crystallization of the present invention in particular also includes precipitation.

Examples for essentially water-miscible or water-soluble organic solvents include: low alkyl alcohols such as methanol, ethanol, propanol and isopropyl alcohol, low alkyl ketones such as acetone and methyl ethyl ketone, low alkyl glycol ethers such as methyl glycol, ethyl glycol, propyl glycol and ethyl diglycol, and dipolar aprotic solvents such as N,N-dimethyl-formamide (DMF), N,N-dimethylacetamide (DMA) and dimethyl sulfoxide (DMSO), including mixtures of these solvents. As particularly preferred examples for the water-miscible organic solvent, acetone and low alkyl alcohols are mentioned. Examples for an organic solvent having limited miscibility or solubility with water include: higher alkyl alcohols such as butanol, iso-butanol amyl alcohol, hexanol, 2-ethylhexanol, benzyl alcohol and cyclohexanol, higher alkyl ketones such as methylbutyl ketone, methyl isobutyl ketone and cyclohexanone, esters such as methyl acetate, ethyl acetate, n-propyl (and isopropyl) acetate, n-butyl (and iso-butyl or sec-butyl) acetate and amyl acetate, ethers such as diethyl ether and diisopropyl ether, chlorinated hydrocarbons such as methylene chloride and chloro-form, acetonitrile and the like, including mixtures of these solvents. Particularly preferred as a solvent having limited miscibility or solubility with water is ethyl acetate.

We have unexpectedly found out that the crystallization of HMG-CoA reductase inhibitors from water-miscible organic solvent like acetone or low alkyl alcohol followed by further recrystallizations with the same solvent can remove only a minor part of nonpolar and a major part of polar impurities, and the crystallization from organic solvent having a limited miscibility with water like ethyl acetate followed by further recrystallizations from the same solvent removed only major nonpolar impurities. The last fact is clearly evident from HPLC diagrams of crude HMG-CoA reductase inhibitor (Fig. 2), HMG-CoA reductase inhibitor after the crystallization from acetone (Fig. 3) and HMG-CoA reductase inhibitor obtained by the crystallization from acetone and further recrystallized from ethyl acetate (Fig. 4). According to this unexpected recognition, the last step of the present invention comprising combined crystallization from water-miscible or water-soluble organic solvent and from an organic solvent having limited miscibility or solubility with water cannot be omitted in the process for achieving HMG-CoA reductase inhibitors of high purity.

The combined crystallization treatment according to the present invention may be effected as follows. First, the crystals of crude HMG-CoA reductase inhibitor are dissolved in the afore-mentioned substantially (preferably 100 %) water-miscible or water-soluble organic solvent, in particular acetone or lower alcohol, and then water is added to let HMG-CoA reductase inhibitor crystallize or precipitate. Alternatively, the crude HMG-CoA reductase inhibitor being dissolved in the substantially water- miscible or water-soluble organic solvent is added to water for being crystallized or precipitated. These procedures may be repeated again with the same or another water-miscible or water-soluble organic solvent, if necessary, for example from one to four times depending on the purity of the starting crude material.

The crystals obtained thereby are then dissolved in the afore-mentioned solvent having limited miscibility or solubility with water, like ethyl acetate, to an appropriate concentration which preferably lies in the range of 10 to 35 g/l, most preferably in the range of 15 to 25 g/l. After the removal of one-third to three-fourth of solvent, the HMG-CoA reductase inhibitor crystallizes. Crystallization from the same or another organic solvent having limited miscibility or solubility with water may be repeated, if necessary, for example for one to three times depending on the purity of the product obtained by crystallization from water-miscible or water-soluble organic solvent. The crystallized HMG-CoA reductase inhibitor is then filtered and dried to yield a product of purity of at least 99.6 %.

As already mentioned, the order of crystallizations may be inverse, i.e. first performing crystallization from the organic solvent having limited miscibility or solubility with water, and then performing crystallization from the water-miscible or water-soluble organic solvent. In a preferred embodiment of the present invention, first performing crystallization from ethyl acetate as the organic solvent having limited miscibility or solubility with water may suitably be effected directly after the ethyl acetate extraction step or, optionally, after the lactonization step described above.

With the process according to the present invention, products having a purity of at least 99.6% and even at least 99.7 % are achievable.

In a further alternative embodiment, the different kinds of crystallizations may be performed repeatedly in an alternating manner.

In another aspect of the present invention, the previously described process of combined crystallization steps from water-

miscible or water-soluble organic solvent and from organic solvent having limited miscibility or solubility with water are employed as a final polishing step of any process for isolation and/or purification of HMG-CoA reductase inhibitors. Accordingly, such a final polishing step can also be applied to raw materials of HMG-CoA reductase inhibitor which have been conventionally obtained. The thus achievable purity of HMG-CoA reductase inhibitor is at least 99.6 % and even at least 99.7 %.

The process according to the present invention is well suited especially when lovastatin is selected as the HMG-CoA reductase inhibitor. Accordingly in another aspect of the present invention, the process described above is used for the isolation and/or purification of lovastatin.

The essentially pure HMG-CoA reductase inhibitors obtained by the process according to the present invention, such as lovastatin, mevastatin, pravastatin and simvastatin as well as their derivatives and analogues, can be beneficially used for the preparation of a pharmaceutical for the prevention and/or treatment of diseases. The obtained inhibitors and pharmaceuticals are particularly useful as medicaments or preventives for reducing the risk of stroke, transient ischemic attack, atherosclerosis and myocardial infarction.

The following examples illustrate the process of the instant invention and are not to be considered as limiting the invention set forth in the claims appended hereto.

***EXAMPLES***

***Example 1***

Fermentation broth (160 l) with concentration of lovastatin 1 g/l obtained by fermentation with *Aspergillus*

*terreus* ATCC 20542 was placed into the vessel (400 l) and adjusted to pH 10 with 1 M aqueous sodium hydroxide solution. After 10 minutes of intensive stirring at room temperature the broth was adjusted to pH 9 with 1 M sulfuric acid solution and the biomass was filtered off. The filtrate was then acidified with 1 M sulfuric acid solution to pH 6.5. 160 l of ethyl acetate was added to filtrate and the obtained mixture was stirred for 20 min. The aqueous and ethyl acetate phases were separated by extraction centrifuge. The ethyl acetate extract was concentrated in rotary evaporator to volume of 14 l. The concentration of the lovastatin in the free acid form in the ethyl acetate concentrate amounted to 10 g/l.

The ethyl acetate concentrate (14 l) was then placed into reactor (40 l) and lactonized. The lactonization was initiated by catalytic amount of TFA (0.5 ml of TFA/ 1 l of concentrate). The lactonization procedure lasted for two hours at 40 °C. The concentrate was washed after the lactonization two times with 14 l of 5 % ammonium hydrogen carbonate aqueous solution. The aqueous phase was discharged, the organic phase was further concentrated to dry in rotary evaporator. The resulted oily product (1.5 l) contained 133 g of lovastatin.

The obtained oily product (161 ml) was dissolved in 80 ml of acetonitrile and loaded on a chromatography column (80 cm, 3.6 cm) filled with XAD-16 (XAD-16 is the commercial name of company Rohm & Hass, 20-50 mesh). The column was eluted first with 40:60 acetonitrile/water (pH 3, adjusted by hydrochloric acid) at a rate of 75 ml/min. Elution was monitored by UV detector (236 nm) and after first drop of absorption the elution of the column with 55:45 acetonitrile/water (pH 3, adjusted by hydrochloric acid) was started. The main fraction was collected and after the fall of the absorption the column was washed with 80:20 acetonitrile/water (pH 3, adjusted by hydrochloric). The acetonitrile was removed from the main fraction by rotary evaporator (50 °C, 150 mbar) and the resulted crystals were filtered off. Mass of crystals was 24.5

g and the content of lovastatin was 50 % weight/weight (w/w). HPLC purity was 92.5 %.

Resulted crystals (24 g) were dissolved in 350 ml acetone and 700 ml water was added under continuos stirring. The mixture was placed on 4 °C for 30 minutes. Obtained crystals were filtered off and dried in vacuo at room temperature. Mass of crystals was 12.7 g with the content 90 % w/w of lovastatin. HPLC purity was 98.8 %.

The crystallization from acetone was repeated under the same condition and 11.3 g of crystals with 97 % w/w of lovastatin were obtained. HPLC purity was 99.4 %.

The crystals (11.3 g) obtained after the second crystallization from acetone were dissolved in 700 ml of ethyl acetate and the ethyl acetate was evaporated in vacuo to the concentration of lovastatin 70 g/l. The concentrate was placed on 8 °C for one hour. Resulted crystals of lovastatin were filtered off and then dried in vacuo. Mass of crystals was 9.4 g with 99.6 % w/w content of lovastatin. HPLC purity was 99.7 %.

***Example 2***

Lovastatin crystals (3 g), isolated after the XAD-adsorption chromatography as described in Example 1, were dissolved in 170 ml ethyl acetate. The ethyl acetate was evaporated in vacuo (200 mbar) at 50 °C to 35 ml. The concentrate was placed on 10 °C for one hour. Resulted crystals of lovastatin were filtered off and then dried in vacuo. Mass of crystals was 2.1 g with 96 % w/w content of lovastatin. HPLC purity was 99.0 %.

The obtained crystals (2.1 g) were dissolved in 50 ml acetone and 85 ml water was added. The mixture was placed then on 10 °C for 30 minutes and the crystals were filtered off and

dried in vacuo at 40 °C. Mass of resulted crystals was 1.9 g with the 99 % w/w of lovastatin. HPLC purity was 99.8 %.

***Example 3***

A fermentation broth (30 l in a 50 l fermentor) containing pravastatin (690 g per kg of fermentation broth; HPLC purity of pravastatin was 48.7 %) was filtered and the resulting mycelium was washed with water. Filtrate (51 l) was acidified to pH 5.0 with 10 % aqueous solution of phosphoric acid. Active substance (pravastatin) was then extracted in an extraction column from the filtrate into 70 l of ethyl acetate. Water phase (50 l) with less than 2 g of pravastatin and with major part of impurities was discharged. The ethyl acetate phase was evaporated to 800 ml and used further in the process of isolation. The HPLC purity of pravastatin in the ethyl acetate extract was 70.3 %.

For further isolation, the oily product was subjected to adsorption chromatography and combined crystallization steps in accordance with Example 1.

***Example 4***

A crude simvastatin (2.3 g) in lactone form was dissolved in acetone (7 ml) and 15 ml of water was added. The result was oily product that crystallized next in 10 minutes. The obtained crystals were then filtered, washed with water and dried at 40 °C for 60 min. The resulted crystals (2.2 g) with HPLC purity of 99.51 % were then dissolved in ethyl acetate (8 ml). The resulted solution was concentrated to 4 ml, and simvastatin was left to crystallize for 60 min at 8 °C. The product was filtered and washed with water. The crystals were then dried at 40 °C for 60 min. The purity of the resulted simvastatin (1.7 g) was 99.73 %.

***Example 5***

A crude mevastatin (2.0 g) in lactone form with HPLC purity 98.5 % was dissolved in acetone (7 ml) and 20 ml of water was added. The result was oily product that crystallized next in 10 min. The obtained crystals were then filtered, washed with water and dried at 40 °C for 60 min. The resulted crystals (1.8 g) with HPLC purity 99.33 % were then dissolved in ethyl acetate (8 ml). The resulted solution was concentrated to 4 ml and mevastatin was left to crystallize for 60 min at 8 °C. The product was filtered and washed with water. The crystals were then dried at 40 °C for 60 min. The purity of the resulted mevastatin (1.3 g) was 99.72 %.

## Claims

1. A process for the isolation and purification of HMG-CoA reductase inhibitors from a mycelium biomass which comprises:
- clarifying a mycelium broth and concentrating the clarified broth to a lower volume,
- acidifying of the concentrate to a pH value in the range of 4.5 to 7.5, followed by extracting the HMG-CoA reductase inhibitor with ethyl acetate,
- optionally performing lactonization,
- crystallization of the HMG-CoA reductase inhibitor from a water-miscible or water-soluble organic solvent, and
- crystallization of the HMG-CoA reductase inhibitor from an organic solvent having limited miscibility or solubility with water.

2. The process according to claim 1, further comprising, before clarifying the mycelium biomass broth, the steps of dissolvating the HMG-CoA reductase inhibitor from a mycelium biomass at pH value between 9.5 and 13 into fermentation liquor, and adjusting the broth to a pH value between 7.5 and 8.5.

3. The process according to claim 2, wherein the dissolvation step is carried out at a temperature in the range of 10 to 40°C for less than one hour.

4. The process according to any one of the preceding claims, wherein clarifying the mycelium broth is carried out by removing the mycelium from the broth by means of filtration.

5. The process according to any one of the preceding claims, wherein said clarified broth is concentrated by means of reverse osmosis.

6. The process according to any one of the preceding claims, wherein the concentrate is acidified to a pH value in the range of 5.5 to 7.5.

7. The process according to claim 6, wherein the concentrate is acidified to a pH value in the range of 6.0 to 7.0.

8. The process according to any one of the preceding claims, wherein the HMG-CoA reductase inhibitor which is extracted from ethyl acetate and optionally lactonized is subjected to a purification step by adsorption chromatography.

9. The process according to claim 8, wherein a mixture of acetonitrile and water is used as the mobile phase for adsorption chromatography.

10. The process according to any one of the preceding claims, wherein the order of the crystallization steps is reversed.

11. The process according to any one of the preceding claims, wherein the water-miscible or water-soluble organic solvent used in the crystallization step is acetone or a low alkyl alcohol.

12. The process according to claim 11, wherein the crystallization step comprises dissolving the HMG-CoA reductase inhibitor in acetone, and then adding water thereto.

13. The process according to any one of the preceding claims, wherein the crystallization step from an organic solvent having limited miscibility or solubility with water comprises dissolving the HMG-CoA reductase inhibitor in said organic solvent at a concentration of 10 to 35 g/l, and removing one-third to three-fourth of said organic solvent.

14. The process according to any one of the preceding claims, wherein the organic solvent having limited miscibility or

solubility with water used in the crystallization step is ethyl acetate.

15. The process according to any one of the preceding claims, wherein HMG-CoA reductase inhibitors are obtained having a purity higher than 99.6%.

16. The process according to any one of the preceding claims, wherein the HMG-CoA reductase inhibitor is selected to be lovastatin.

17. A process for the purification of HMG-CoA reductase inhibitors which comprises subjecting the HMG-CoA reductase inhibitor to combined crystallization steps comprising crystallization from an water-miscible or water-soluble and crystallization from an organic solvent having miscibility or solubility with water.

18. The process according to claim 17, wherein the combined crystallization steps are conducted as final polishing steps to obtain HMG-CoA reductase inhibitors having a purity higher than 99.6%.

19. The process according to claim 18, wherein the obtained HMG-CoA reductase inhibitors have a purity higher than 99.7%.

20. The process according to any one of claims 17 to 19, wherein acetone or a low alkyl alcohol is used as the water-miscible or water-soluble organic solvent.

21. The process according to claim 20, wherein said crystallization comprises dissolving the HMG-CoA reductase inhibitor in acetone, and then adding water thereto.

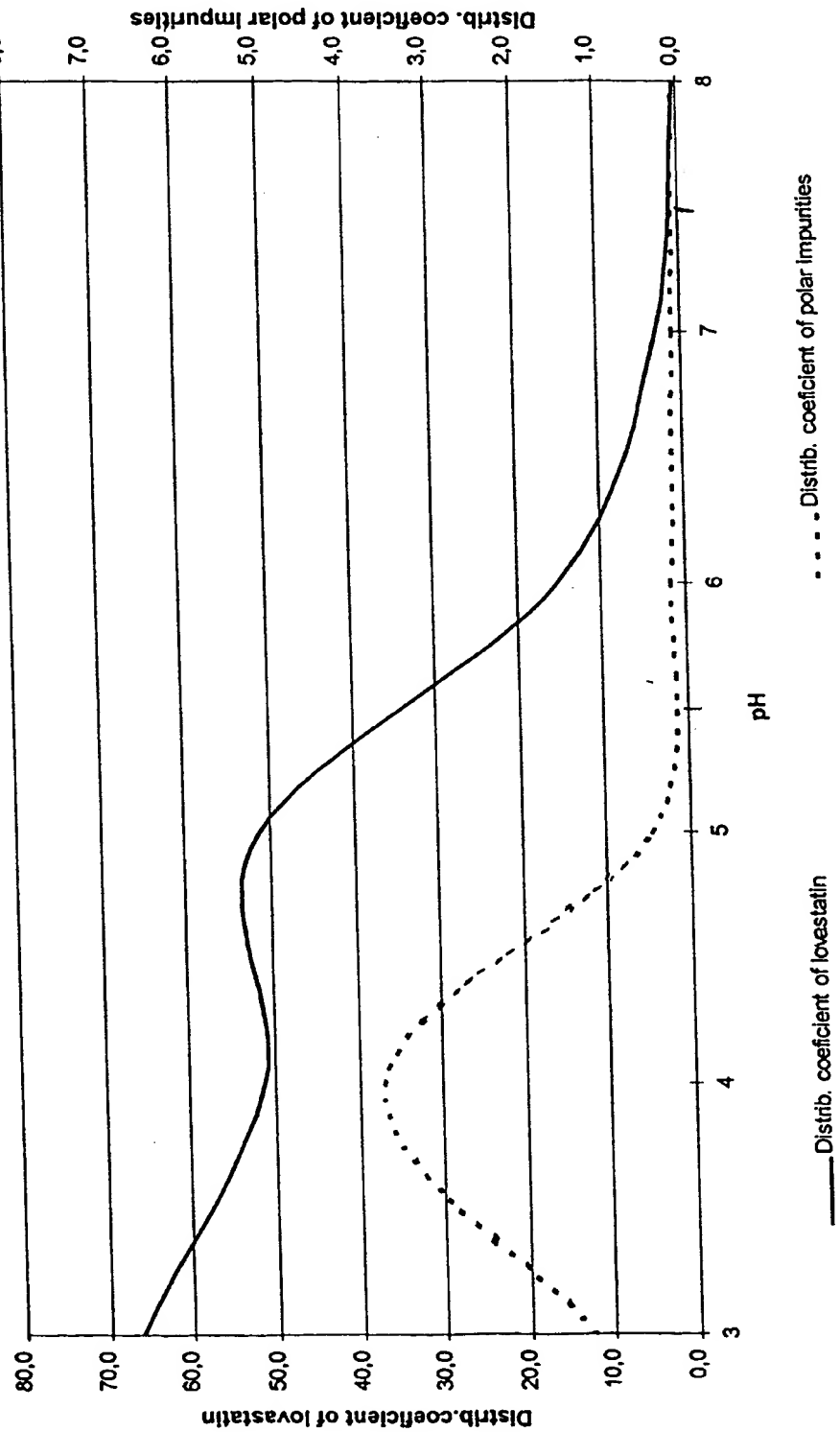
22. The process according to any one of claims 17 to 21, wherein said crystallization from said organic solvent having limited miscibility or solubility with water comprises

dissolving the HMG-CoA reductase inhibitor in said organic solvent at a concentration of 10 to 35 g/l, and removing one-third to three-fourth of said organic solvent.

23. The process according to any one of claims 17 to 22, wherein ethyl acetate is used as the organic solvent having limited miscibility or solubility with water.

24. Use of a process according to claim 1 or a process according to claim 17 for the isolation and/or purification of lovastatin.

Fig.1


Distrib.coefficient of lovastatin
80,0
70,0
60,0
50,0
40,0
30,0
20,0
10,0
0,0
3
4
5
pH
6
7
8
Distrib. coefficient of polar impurities
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
Distrib. coeficient of lovastatin
Distrib. coeficient of polar impurities

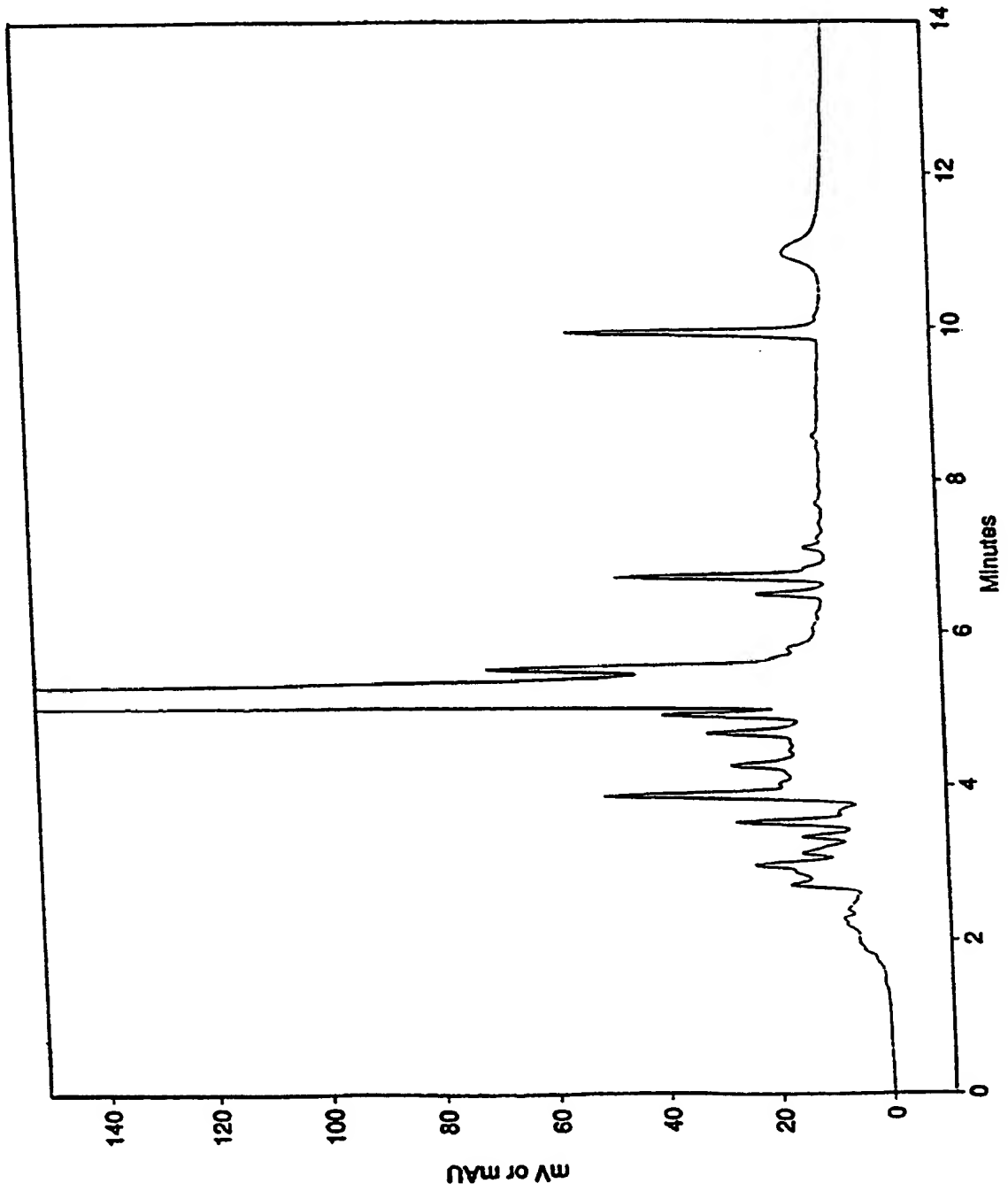
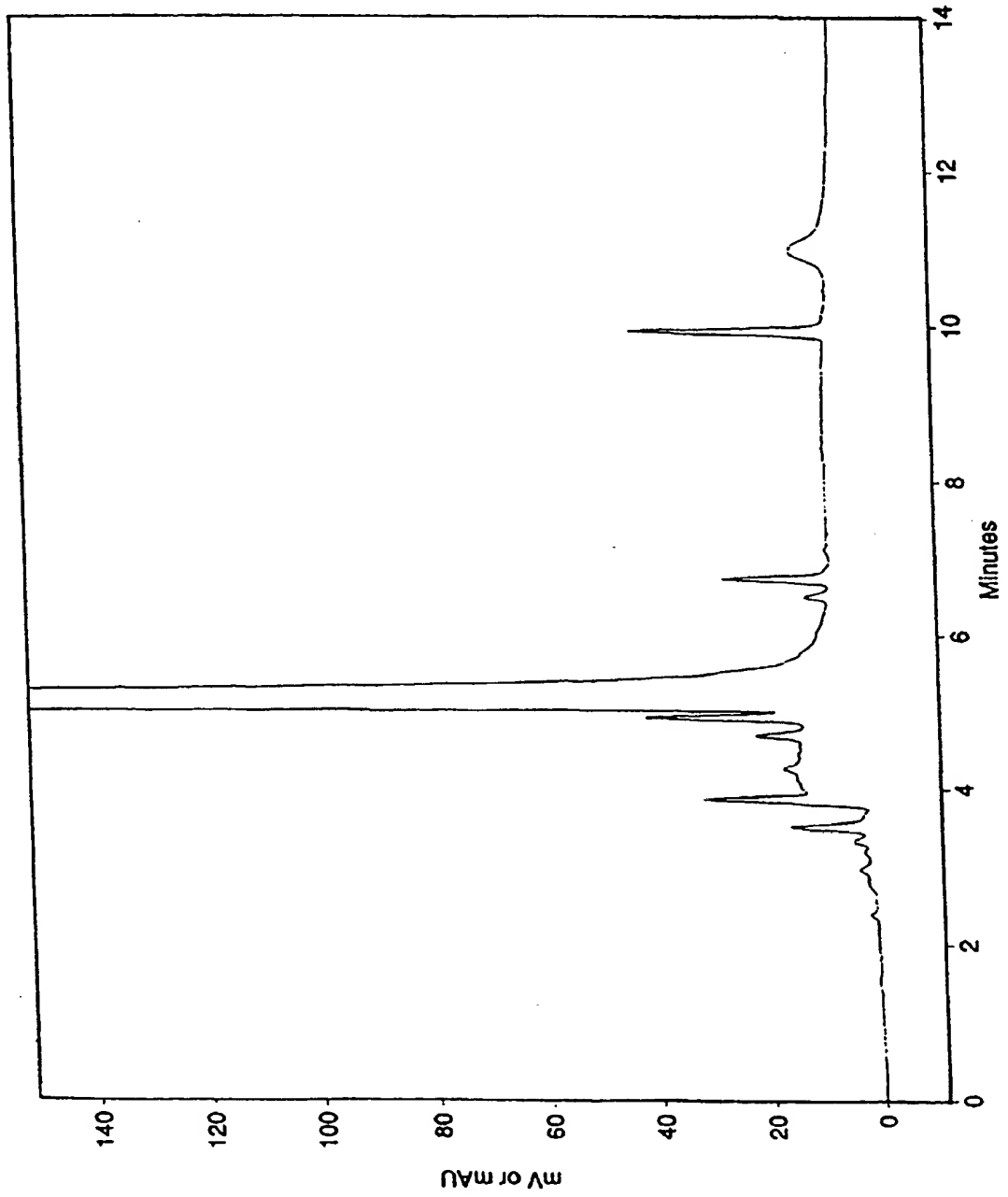



Fig.2

Fig.3



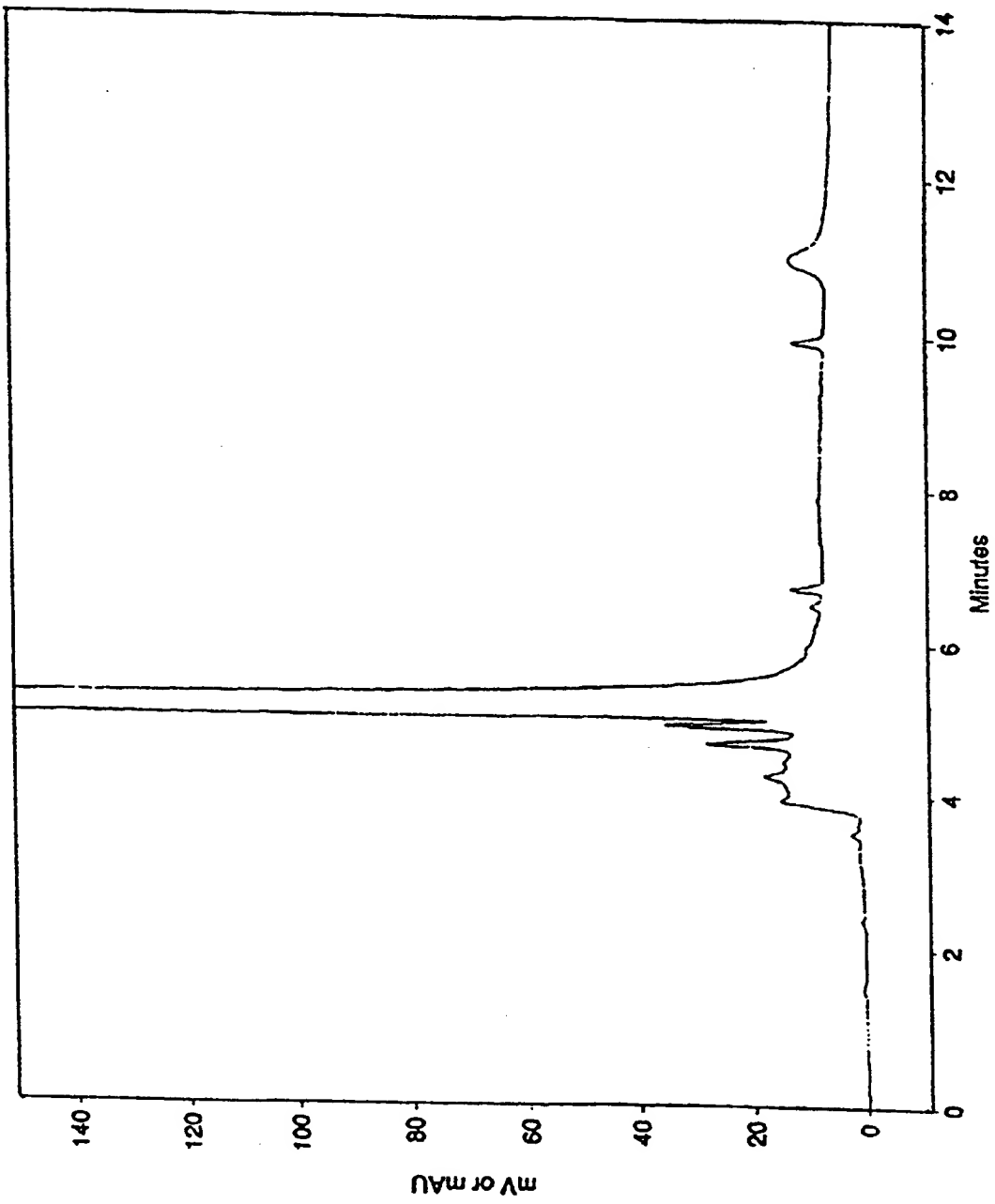



Fig.4

TBK

TIED[redacted]E - BÜHLING - KINNE[redacted] PARTNER (GbR)

TBK-Patent POB 20 19 18 80019 München

An das
Europäische Patentamt

80298 München

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Dipl.-Ing. Olaf Ungerer
Dipl.-Ing. Jürgen Feldmeier
Dipl.-Ing. Stefan Klingele
Dipl.-Chem. Stefan Bühling

January 24, 2000

PCT Patent Application No.: PCT/IB99/00808
LEK PHARMACEUTICAL AND CHEMICAL CO., D.D. et al.
Our ref.: WO 21555

This is in reply to the Official Communication pursuant to Rule 66 PCT dated November 16, 1999.

1. For further examination proceedings enclosed are filed claim sheet replacement pages 17 and 18 to replace the corresponding pages 17 and 18 of the application as originally filed.

By means of the amended claim sheets, original Claim 17 has been amended by incorporating the feature stated in the original Claim 18. Furthermore, thanks to the additional note under section VI of the above referenced Communication, the word "limited" has been inserted between "organic solvent having" and "miscibility or solubility" (note that the word "limited" was not missing in original Claim 22 which corresponds to the present Claim 21). Besides, support for this correction of Claim 17 can be found on description page 10, lines 1-4.

Deutsche Bank München Kto. 286 1060 BLZ 700 700 10
Dresdner Bank München Kto. 3939 844 BLZ 700 800 00
Postbank München Kto. 67043 804 BLZ 700 100 80
Dai-Ichi-Kangyo Bank München Kto. 51 042 BLZ 700 207 00
Sanwa Bank Düsseldorf Kto. 500 047 BLZ 301 307 00

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GG 1/4

2. Novelty

Concerning the basic technical concept of the present invention as defined by the valid Claim 17, it is noted that none of the documents D1 (WO-A-97/20834), D2 (US-A-4319039) and D3 (US-A-4294846) disclose the procedure measure of subjecting HMG-CoA reductase inhibitor to a combined crystallization step, which comprises crystallization from a water-miscible or water-soluble and crystallization from an organic solvent having limited miscibility or solubility with water, as a final polishing step to obtain HMG-CoA reductase inhibitors having a purity higher than 99.6 %.

In D1 a specific method for purification of lovastatin is described which comprises successive recrystallizations from a mixture of nitrile/water a mixture of a low alcohol acetate/$C_7$-$C_9$ alkane and from acetone (with the intermittent decoloring step by means of aluminum oxide and activated carbon), thereby achieving a HPLC purity of 99,5 % (see Claim 1 and pages 2 and 3 of D1). However, it was not recognized in D1, nor is it desirable from this document that by the presently claimed combined crystallization steps, where HMG-CoA reductase inhibitor is subsequently subjected to the specific combined crystallization steps as final polishing steps, a purity higher than 99.6 % is obtained.

Likewise, neither the D2 (columns 4 and 13), nor the D3 (columns 7-8) specifically disclose the presently claimed combined crystallization steps as final polishing steps to achieve the claimed purity of higher than 99.6 %.

Referring to the subject matter of the present Claim 1, the above observations even more apply. Specifically, the

process steps additionally recited in Claim 1, especially the acidifying of the clarified brooth concentrate to the particular pH value range of 4.5 to 7.5 and the subsequent ethyl acetate extracting step, are neither taught nor suggested by the cited references.

Hence, the subject matters of original Claim 1 and of valid Claim 17 are considered to be novel over the cited prior art.

3. Inventive step

As the present inventors have recognized that subsequent combination of specific crystallization steps as final polishing steps achieves a purity of a HMG-CoA reductase inhibitor in a range higher than 99.6 %, the present invention clearly involves an inventive merit against the prior art. In this connection, it is noted that a HMG-CoA reductase inhibitor purity higher than 99.6 % is very difficult to achieve, because undesired side products tend to be very similar in structure to the desired effective inhibitor. Therefore, conventional purification schemes, sometimes including crystallizations from various solvents, may not lead to a significant purification success but may adversely lead to a significant loss of yield. On the other hand, the specific combined crystallization steps according to the present invention lead to a significant improvement of purity to a range higher than 99.6 % when performed as a final polishing step, as demonstrated by the examples of the present application. The process of Claim 17 is particularly useful to further purify crude HMG-CoA reductase inhibitors, as shown in the present examples 4 and 5.

Therefore, the subject matter defined in the valid Claim 17 also involves an inventive step.

It is to be further emphasized that the prior art does not teach or suggest the beneficial effect being associated with the additional process features stated in the present Claim 1, especially the ethyl acetate extraction step of a HMG-CoA reductase inhibitor-containing concentrate. For this effect to be achieved, the previous adjustment of the pH value to lie within the range of 4.5 to 7.5 is very significant. These features and effects are further explained, for example, in the first paragraph of page 5 and demonstrated by Fig. 1 of the present application.

Therefore, it is respectfully asked to communicate an approving statement not only for novelty, but also for inventive step of the present Claims 1 and 17.

Dr. Andreas Oser
Patentanwalt
***Tiedtke-Bühling-Kinne***

Enclosures:
- Claim sheet replacement pages 17 and 18

solubility with water used in the crystallization step is ethyl acetate.

15. The process according to any one of the preceding claims, wherein HMG-CoA reductase inhibitors are obtained having a purity higher than 99.6%.

16. The process according to any one of the preceding claims, wherein the HMG-CoA reductase inhibitor is selected to be lovastatin.

17. A process for the purification of HMG-CoA reductase inhibitors which comprises subjecting the HMG-CoA reductase inhibitor to combined crystallization steps, which comprises crystallization from an water-miscible or water-soluble and crystallization from an organic solvent having limited miscibility or solubility with water, as final polishing steps to obtain HMG-CoA reductase inhibitors having a purity higher than 99.6%.

18. The process according to claim 17, wherein the obtained HMG-CoA reductase inhibitors have purity higher than 99.7 %.

19. The process according to claim 1 or 18, wherein wherein acetone or a low alkyl alcohol is used as the water-miscible or water-soluble organic solvent.

20. The process according to claim 19, wherein said crystallization comprises dissolving the HMG-CoA reductase inhibitor in acetone, and then adding water thereto.

21. The process according to any one of claims 17 to 20, wherein said crystallization from said organic solvent having limited miscibility or solubility with water comprises dissolving the HMG-CoA reductase inhibitor in said organic solvent at a concentration of 10 to 35 g/l, and removing one-third to three-fourth of said organic solvent.

22. The process according to any one of claims 17 to 21, wherein ethyl acetate is used as the organic solvent having limited miscibility or solubility with water.

23. Use of a process according to claim 1 or a process according to claim 17 for the isolation and/or purification of lovastatin.

FB

PATENT COOPERATION TREATY

# PCT

09/600566

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| Applicant's or agent's file reference | FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below. | |
|---|---|---|
| WO 21555 | | |
| International application No. | International filing date *(day/month/year)* | (Earliest) Priority Date *(day/month/year)* |
| PCT/IB 99/ 00808 | 17/02/1999 | 18/02/1998 |
| Applicant | | |
| LEK PHARMACEUTICAL AND CHEMICAL...et al. | | |

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

[X] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[ ] None of the figures.

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 99/00808

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12P17/06 C12P7/42 C12P7/62 C07D309/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12P C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | WO 97 20834 A (ANTIBIOTIC CO ;DIMOV DIMCHO IVANOV (BG); GROZDANOV GEORGY ASENOV ()) 12 June 1997 (1997-06-12) cited in the application the whole document | 1-24 |
| Y | WO 97 06128 A (GIST BROCADES BV ;PATER ROBERTUS MATTHEUS DE (NL); SIBEYN MIEKE (N) 20 February 1997 (1997-02-20) cited in the application the whole document | 1-24 |
| Y | US 4 319 039 A (ALBERS-SCHONBERG GEORGE) 9 March 1982 (1982-03-09) column 4, line 37 - line 60; claims | 1-24 |

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 14 July 1999 | 20/07/1999 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Delanghe, L |

Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

International Application No
[redacted]/IB 99/00808

C.(Continuation) DOCUMENTS CONSIDE[redacted]TO BE RELEVANT

| Category ° | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | US 4 965 200 A (CHEN SHIEH-SHUNG J ET AL) 23 October 1990 (1990-10-23) column 8, paragraph 1; claims | 1-24 |
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| Y | EP 0 251 625 A (MERCK & CO INC) 7 January 1988 (1988-01-07) claims | 1 |

1

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Information on patent family members

International Application No
IB 99/00808

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
IB 99/00808

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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# PATENT COOPERATION TREATY

09/600566

From the INTERNATIONAL BUREAU

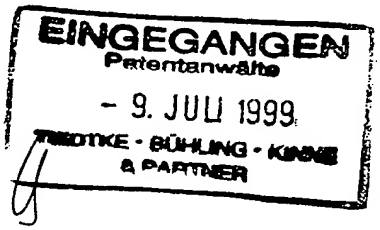
## PCT

### NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

OSER, Andreas
Bavariaring 4
D-80336 München
ALLEMAGNE




| | |
|---|---|
| Date of mailing (day/month/year)<br>30 June 1999 (30.06.99) | |
| Applicant's or agent's file reference<br>WO 21555 | **IMPORTANT NOTIFICATION** |
| International application No.<br>PCT/IB99/00808 | International filing date (day/month/year)<br>17 February 1999 (17.02.99) |
| International publication date (day/month/year)<br>Not yet published | Priority date (day/month/year)<br>18 February 1998 (18.02.98) |
| Applicant<br>LEK PHARMACEUTICAL AND CHEMICAL COMPANY D.D. et al | |

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

| Priority date | Priority application No. | Country or regional Office or PCT receiving Office | Date of receipt of priority document |
|---|---|---|---|
| 18 Febr 1998 (18.02.98) | P-9800046 | SI | 17 June 1999 (17.06.99) |

**The International Bureau of WIPO**
**34, chemin des Colombettes**
**1211 Geneva 20, Switzerland**

Facsimile No. (41-22) 740.14.35

Authorized officer

Ting Zhao

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

# PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

| | |
|---|---|
| **Date of mailing** (day/month/year)<br>18 November 1999 (18.11.99) | |
| **International application No.**<br>PCT/IB99/00808 | **Applicant's or agent's file reference**<br>WO 21555 |
| **International filing date** (day/month/year)<br>17 February 1999 (17.02.99) | **Priority date** (day/month/year)<br>18 February 1998 (18.02.98) |
| **Applicant**<br>PFLAUM, Zlatko et al | |

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

15 September 1999 (15.09.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

| | |
|---|---|
| **The International Bureau of WIPO**<br>**34, chemin des Colombettes**<br>**1211 Geneva 20, Switzerland** | Authorized officer<br>Ingrid Aulich |
| Facsimile No.: (41-22) 740.14.35 | Telephone No.: (41-22) 338.83.38 |

# PATENT COOPERATION TREATY

09/600566

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
OSER, Andreas
Tiedtke-Bühling-Kinne
Bavariaring 4
D-80336 München
ALLEMAGNE

# PCT

## WRITTEN OPINION

(PCT Rule 66)

| | |
|---|---|
| Date of mailing *(day/month/year)* | 16. 11. 99 |
| Applicant's or agent's file reference: WO 21555 | **REPLY DUE** **within 3 month(s)** from the above date of mailing |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/IB99/00808 | 17/02/1999 | 18/02/1998 |

International Patent Classification (IPC) or both national classification and IPC
C12P17/06

Applicant
LEK PHARMACEUTICAL AND CHEMICAL... et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

| | | |
|---|---|---|
| I | ☒ | Basis of the opinion |
| II | ☐ | Priority |
| III | ☐ | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| IV | ☐ | Lack of unity of invention |
| V | ☒ | Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| VI | ☐ | Certain document cited |
| VII | ☐ | Certain defects in the international application |
| VIII | ☒ | Certain observations on the international application |

3. The applicant is hereby **invited to reply** to this opinion.

**When?** See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

**How?** By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

**Also:** For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

**If no reply is filed**, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 18/06/2000.

16.2.001

| | |
|---|---|
| Name and mailing address of the international preliminary examining authority:<br>European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Authorized officer / Examiner<br>Korsner, S-E<br>Formalities officer (incl. extension of time limits)<br>DA ROCHA, O.<br>Telephone No. +49 89 2399 8101 |

Form PCT/IPEA/408 (cover sheet) (January 1994)

## I. Basis of the opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*):

**Description, pages:**

1-14 as originally filed

**Claims, No.:**

1-24 as originally filed

**Drawings, sheets:**

1/4-4/4 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

## V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

| | | |
|---|---|---|
| Novelty (N) | Claims | to be settled |
| Inventive step (IS) | Claims | to be settled |
| Industrial applicability (IA) | Claims | 1-24...Yes |

2. Citations and explanations

**see separate sheet**

**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

**see separate sheet**

## V. Reasoned statement

The following documents will be referred to in this opinion:

D1 = WO - A - 9720834
D2 = US - A - 4 319 039
D3 = US - A - 4 294 846

1. Novelty (Article 33(2) PCT)

On pages 2-3 of D1, it is stated that the raw lovastatin can be recrystallized, e.g. in acetate of low-alcohol-alkane C7-C9 (!?) and then in acetone; the former appears to have a limited miscibility and the latter a high one -> thus being novelty-destroying for the claimed scope, at least in the broadest sense (Claim 17).

D2 (columns 4 and 13) and D3 (columns 7-8) also refer to repeated crystallizations where solvents of different miscibility with water may be used.

2. Inventive step (Article 33(3) PCT)

I.
Although the prior art does not provide a particular teaching about the usefulness of recrystallization, it cannot be excluded that there was a certain knowledge (but not optimized) in the art about the advantage of using different solvents (as in D1-D3).

In that case, the present process may possibly be seen as an improved alternative (to be discussed) and must then rely on further definitions in

order to be distinguished from the processes of the art.

II.
Moreover, an objection could be raised on the ground that the alleged improvement in purity may only be achieved under certain circumstances. For instance, the mere recrystallization (of Claim 17) may give a result which is much inferior to that of Claim 1 (which includes further steps), in particular when carried out with non-preferred combinations of solvents.

The Applicant is invited to submit further information about the inventive step and to discuss whether the alleged purity can be generally obtained without further optimization (i.e. with no further essential features).

## VIII. Certain observations

1.
The scope of Claim 17 is much broader than that of Claim 1, which latter includes the additional definitions concerning pH-adjustment and ethyl acetate extraction.
The claim should therefore rather precede Claim 1 (in case patentability can be established - see above).

NB.
It appears that the word "limited" is missing before "miscibility" at the end of Claim 22; the claim would otherwise cover repeated crystallization from solvents which may be identical (?).
Compare Claim 22, which seems to indicate that "limited" is missing.

***

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## Correspondence with the EPO on PCT Chapter II demands

In order to ensure that your PCT Chapter II demand is dealt with as promptly as possible you are requested to use the enclosed self-adhesive labels with any correspondence relating to the demand sent to the Munich Office.

One of these labels should be affixed to a prominent place in the upper part of the letter or form etc. which you are filing.

PATENT COOPERATION TREATY



# PCT

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| WO 21555 | | |
| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
| PCT/IB99/00808 | 17/02/1999 | 18/02/1998 |
| International Patent Classification (IPC) or national classification and IPC | | |
| C12P17/06 | | |
| Applicant | | |
| LEK PHARMACEUTICAL AND CHEMICAL... et al. | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 15/09/1999 | 17.04.2000 |
| Name and mailing address of the international preliminary examining authority: | Authorized officer |
| European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Korsner, S-E<br>Telephone No. +49 89 2399 8554 |

Form PCT/IPEA/409 (cover sheet) (January 1994)

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IB99/00808

## I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

**Description, pages:**

| | | | | | |
|---|---|---|---|---|---|
| 1-14 | as originally filed | | | | |

**Claims, No.:**

| | | | | | |
|---|---|---|---|---|---|
| 1-13,14 (part) | as originally filed | | | | |
| 14 (part),15-23 | as received on | 24/01/2000 | with letter of | 24/01/2000 | |

**Drawings, sheets:**

| | |
|---|---|
| 1/4-4/4 | as originally filed |

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

## V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 1-23 |
| | No: | Claims | |
| Inventive step (IS) | Yes: | Claims | 1-23 |
| | No: | Claims | |
| Industrial applicability (IA) | Yes: | Claims | 1-23 |
| | No: | Claims | |

2. Citations and explanations

**see separate sheet**

## VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

**see separate sheet**

## V. Reasoned statement

The following documents will be referred to in this report:

D1 = WO - A - 9720834
D2 = US - A - 4 319 039
D3 = US - A - 4 294 846

1. Novelty (Article 33(2) PCT)

The subject-matter of the amended claims is novel over the prior art.
[An initial objection concerning Claim 17 has been withdrawn because the amended claim includes a definition of the degree of purity.]

2. Inventive step (Article 33(3) PCT)

On pages 2-3 of D1, it is stated that the raw lovastatin can be recrystallized, e.g. in acetate of low-alcohol-alkane C7-C9 (!?) and then in acetone; the former appears to have a limited miscibility with water and the latter a high one.
D2 (columns 4 and 13) and D3 (columns 7-8) also refer to repeated crystallizations where solvents of different miscibility with water may be used.

However, it is considered that the prior art does not provide a clear teaching towards the present invention.

The presence of an inventive step is therefore acknowledged for the claimed scope.

## VIII. Certain observations

1.
The scope of Claim 17 is broader than that of Claim 1, which latter includes additional definitions concerning pH-adjustment and ethyl acetate extraction. The claim should therefore rather precede Claim 1.

2.
The amended Claim 17 includes a degree of purity ("desideratum"), which may require a preferred combination of solvents.
No objection has been raised to this.

The amended claim refers also to the "final polishing steps"; this has no explicit basis in the Description but may be deduced from the Examples.

3.
Note a minor error on page 11, lines 26-27 (...name of a resin sold by the...?)

- - - - -

PATENT COOPERATION TREATY

09/600566

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ERNATIONAL PRELIMINARY EXAMINING AUTHORITY

# PCT

To:

OSER, Andreas
Tiedtke-Bühling-Kinne
Bavariaring 4
D-80336 München
ALLEMAGNE



NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)* 17.04.2000

Applicant's or agent's file reference
WO 21555

**IMPORTANT NOTIFICATION**

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/IB99/00808 | 17/02/1999 | 18/02/1998 |

Applicant
LEK PHARMACEUTICAL AND CHEMICAL... et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

| Name and mailing address of the IPEA/ | Authorized officer |
|---|---|
| European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Gallego, A<br><br>Tel.+49 89 2399-8102 |

Form PCT/IPEA/416 (July 1992)